




  
**Knowledge E**  
Engaging minds

 **KnE Life Sciences**

## The Veterinary Medicine International Conference (VMIC)

12-14 July 2017

ISSN 2413-0877



[www.KnEpublishing.com](http://www.KnEpublishing.com)

Search articles, journals, authors and more...

ALL

SEARCH

## Latest Articles

<https://knepublishing.com/index.php/Kne-Life/index>

## Browse All Issues

<https://knepublishing.com/index.php/Kne-Life/issue/archive>

## The Veterinary Medicine International Conference (VMIC)

VMIC—The Veterinary Medicine International Conference—is one of the world's leading conference focusing on a wide array of topics including Veterinary Medicine and Biomedical Science. It offers a stimulating venue for scientists, researchers, lecturers, general practitioners, and others to broaden their social scientific network. This conference aimed at improving human and public health by improving agricultural and food systems, advancing biomedical and comparative medical research, preventing and addressing zoonotic diseases, kit diagnostic, enhancing environmental and ecosystem health, and helping manage the 21st-century public health challenges.

Conference date: 12–14 July 2017

Location: Surabaya, East Java, Indonesia

Editors: Sri Agus Sudjarwo, Fedik A. Rantam, rer. nat. Gunawan Indrayanto, Muchammad Yunus, Rimayanti, Wiwik Misaco, Tita Damayanti Lestari, Mustofa Helmi Effendi, Dikky Eka Mandala Putranto, and Shafia Khairani

Organizer: Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia

Sponsors: Ministry of Research, Technology and Higher Education of the Republic of Indonesia, Universitas Airlangga, ROMINDO

PRIMAVENTCOM, Gadjah Mada University, Bogor Agriculture Institute, ILRI (International Livestock Research Institute), Kagoshima University, Miyazaki University, Faculty of Veterinary Science, Chulalongkorn University, College of Veterinary Medicine, Tarlac Agricultural University, Yamaguchi University, Erasmus MC, Kasetsart University, Wyndham Veterinary Clinic, GERBU – Germany, USAID

Published: 29 November 2017

ISSN: 2413-0877

## TABLE OF CONTENTS

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 1-9Toll-Like Receptors (TLRs) Play Role in Adaptive Immunity in Rabbits Immunized by *Sarcoptes scabiei* Proteins<https://knepublishing.com/index.php/Kne-Life/article/view/1100/2856>

Nunuk Dyah Retno Lastuti, Fedik Abdul Rantam, Poedji Hastutie, Dony Chrismanito

[DOWNLOAD FULLTEXT](https://knepublishing.com/index.php/Kne-Life/article/download/1100/2855) (<https://knepublishing.com/index.php/Kne-Life/article/download/1100/2855>)
The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 10-20Preservation Effect of Grouper (*Epinephelus* sp) Fillet Against Survival of Anisakidae<https://knepublishing.com/index.php/Kne-Life/article/view/1101/2858>

Hartanto M. Raharjo, Setiawan Koesdarto, A.T Soelih Estoepangestie, Kusuma Wardhani LD

[DOWNLOAD FULLTEXT](https://knepublishing.com/index.php/Kne-Life/article/download/1101/2857) (<https://knepublishing.com/index.php/Kne-Life/article/download/1101/2857>)
The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 21-27Acanthocephalan in *Xenochrophis piscator* Snake in Sidoarjo Indonesia<https://knepublishing.com/index.php/Kne-Life/article/view/1102/2860>

Inggarsetya Syah Audini, Lucia Tri Suwanti, Setiawan Koesdarto, Emmanuel Djoko Poetranto

[DOWNLOAD FULLTEXT](https://knepublishing.com/index.php/Kne-Life/article/download/1102/2859) (<https://knepublishing.com/index.php/Kne-Life/article/download/1102/2859>)
The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 28-33The Identification Blood Parasites On Pig (*Susdomesticus*) In Polewali Mandar District<https://knepublishing.com/index.php/Kne-Life/article/view/1103/2862>

Silvana Arfin, Lucia Muslimin, Adryani ris



[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1103/2861>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 34-40

### Spirometra in Ptyas mucosus Snake in Sidoarjo, Indonesia

(<https://knepublishing.com/index.php/Kne-Life/article/view/1104/2864>)

Garindra Tiara Pranashinta, Lucia Tri Suwanti, Setiawan Koesdarto, Emmanuel Djoko Poetranto

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1104/2863>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 41-47

### Toxicity of Citrus mitis, Citrus aurantifolia, and Citrus maxima leaf extract toward mortality of Aedes aegypti larvae (Diptera: Culicidae)

(<https://knepublishing.com/index.php/Kne-Life/article/view/1105/2866>)

Hamidah Hamidah, Hebert Adrianto

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1105/2865>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 48-61

### An In Vitro Antibacterial Activity Test of Meniran Herbs' (Phyllanthus Niruri L.) Ethanol Extract Against Mycoplasma gallisepticum causes Chronic Respiratory Disease (CRD) in Broiler Chickens

(<https://knepublishing.com/index.php/Kne-Life/article/view/1106/2868>)

Emy Koestanti Sabdoningrum, Sri Hildanah, Retno Sri Wahjuni, Sri Chusniati, Arimbi Arimbi

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1106/2867>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 62-68

### Bioremediation of Mercury (II) Contaminated Seawater Using the Diatom Skeletonema costatum

(<https://knepublishing.com/index.php/Kne-Life/article/view/1115/2870>)

Thain Soedarti, Timi S., Supripto H., Eko P. Kuncoro

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1115/2869>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 69-76

### BMP-2 Expression of Post Tooth Extraction that Catfish Oil Application

(<https://knepublishing.com/index.php/Kne-Life/article/view/1116/2872>)

Theresia Indah B., Bambang Sumaryono, Ketut Suardita, Amelia Putri R.

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1116/2871>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 77-83

### Inhibition of Apoptosis in Retinal of Newborn Mice Due to Congenital Toxoplasmosis

(<https://knepublishing.com/index.php/Kne-Life/article/view/1117/2874>)

Lucia Tri Suwanti, Mufasirin Mufasirin, Hani Plumeriastuti

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1117/2873>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 84-92

### Effect of Spirulina Platensis on The Number of Spermatogenic Cells in The Seminiferous Tubules of Rat (Rattus Norvegicus) with Excessive Physical Exercise

(<https://knepublishing.com/index.php/Kne-Life/article/view/1118/2876>)

Rahmah Wahyu Rosidawati, Rimayanti Rimayanti, Koesnoto Supranianondo

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1118/2875>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 93-104

### Phytochemicals, Antioxidant and Antifungal Properties of Acorus calamus, Curcuma mangga, and Allium sativum

(<https://knepublishing.com/index.php/Kne-Life/article/view/1119/2878>)

Bayyinatul Muchtaromah, Mujahidin Ahmad, Emy Koestanti S, Yuni Ma'rifatul A, Velayati Labone A

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1119/2877>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 105-110

### **Shark Species on Export Products from East Java and Bali by Dna Barcoding Based on Internal Transcribed Spacer-2 (Its-2) Locus in Mitochondrial**

(<https://knepublishing.com/index.php/Kne-Life/article/view/1120/2880>)

Eduardus Birno Aksono

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1120/2879>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 111-124

### **Bioactivity of human Menopausal Gonadotrophin (hMG) and Deglycosylated hMG (hMGdG) from Urine of Post-Menopausal Women On invitro Bovine Embryonic cleavage**

(<https://knepublishing.com/index.php/Kne-Life/article/view/1121/2882>)

Herry Agoes Hermadiv

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1121/2881>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 125-138

### **The Effect of Frequency Acoustic Stimulation Sound on Intrauterine Weakening of Pregnant Sheep**

(<https://knepublishing.com/index.php/Kne-Life/article/view/1122/2884>)

Djamil Suherman, Hermanto Tri Joewono, I Komang Wiarsa Sardjana

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1122/2883>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 139-152

### **The Potency of $\Delta F$ 508-T Gen Mutant the Coding of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) As Prototype at the Congenital Bilateral Absence of Vas Deferens (CBAVD) Disease in Indonesia**

(<https://knepublishing.com/index.php/Kne-Life/article/view/1123/2886>)

Maslichah Mafruchati, Mas'ud Harijadi, Widjati Widjati, Boerhan Hidayat

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1123/2885>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 153-165

### **A Retrospective Study of Canine Pyometra in Segar Veterinary Hospital, Kuala Lumpur, Malaysia Year 2012-2016**

(<https://knepublishing.com/index.php/Kne-Life/article/view/1124/2888>)

Ng Xin Hul, Mas'ud Hariadi, Hardany Primartiky

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1124/2887>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 166-174

### **Oocyte Quality and Subsequent In Vitro Maturation of Sheep Oocyte-Cumulus Complex from Ovary with Presence and Absence of Corpus Luteum**

(<https://knepublishing.com/index.php/Kne-Life/article/view/1125/2890>)

Rini Widyastuti, Mas Rizky A.A. Syamsunarno, Takdir Salli, Arief Boediono

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1125/2889>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 175-188

### **The Effect of Insulin-Like Growth Factor-I of Pregnant Crossbred Mare Serum and Insulin-Like Growth Factor-I Recombinant Mouse on Estrous Cycles and Litter Sizes of Mice (*Mus musculus*)**

(<https://knepublishing.com/index.php/Kne-Life/article/view/1126/2892>)

Tjuk Imam Restiadi, Imam Mustofa, Suzanita Utama, Sri Mulyati

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1126/2891>)



The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 189-196

### Adding of L-Arginin Amino Acidin Skim Milk Diluent to Maintain Quality of Buck Sperm in Cold Temperature

(<https://knepublishing.com/index.php/Kne-Life/article/view/1127/2894>)

Tri Wahyu Suprayogy, Suherni Susilowati, Tatik Hernawati

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1127/2893>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 197-204

### Profile of Crude Protein Tyrosine Kinase on Plasma Membrane of Merino Sheep Spermatozoa Using the Method of SDS-Page (Sodium Dodecyl Sulphate-Polyacrilamide Gel Electrophoresis)

(<https://knepublishing.com/index.php/Kne-Life/article/view/1128/2896>)

Vilda Carlenia Wardani, Sri Partja Madyawati, Poedji Hastutiek

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1128/2895>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 205-211

### Comparison of Morula and Blastula Embryo Vitricification by Using Cryoprotectant Ethylene Glycol, Propanediol, DMSO and Insulin Transferrin Selenium

(<https://knepublishing.com/index.php/Kne-Life/article/view/1129/2898>)

Widjiati Widjiati, Epy Muhammad Luqman, Portia Sumarsono

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1129/2897>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 212-223

### Effect of L-Arginine on the Thickness Iliac Arteries Wall Post Fogarty Balloon Embolectomy Catheter in Rabbit (*Oryctolagus cuniculus*)

(<https://knepublishing.com/index.php/Kne-Life/article/view/1130/2900>)

Gavrila Amadea Puspitarani, Ngakan Made Rai Widjaja, Hardany Primarizky

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1130/2899>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 224-233

### Ellagic Acid Activity in Healing Process of Incision Wound on Male Albino Rats (*Rattus norvegicus*)

(<https://knepublishing.com/index.php/Kne-Life/article/view/1131/2902>)

Hardany Primarizky, Wiwik Misaco Yuniarti, Bambang Sektiari Lukiswanto

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1131/2901>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 234-240

### Control and Preventive Study of Brucellosis by Using

(<https://knepublishing.com/index.php/Kne-Life/article/view/1132/2904>)

Rahmahani J, Handijatno D, Tyaningsih W, Suwarno Suwarno

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1132/2903>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 241-251

### Hispatology of Coronary artery of male rat (*Ratus Norvegicus*) with high fat diet after being given ethanol extract of Indian acalypha (*Acalypha indica*. L)

(<https://knepublishing.com/index.php/Kne-Life/article/view/1133/2906>)

Kurnijasanti R, Winarti D, Wahyuni R.S., Puguh K, Setyabudi Setyabudi, Sukmanadi Sukmanadi, Sugihartuti R, Damayanti R, Hidayati N, Rahmawati K, Sudjarwo A.S.

[DOWNLOAD FULLTEXT](#) (<https://knepublishing.com/index.php/Kne-Life/article/download/1133/2905>)

The Veterinary Medicine International Conference (VMIC) (<https://knepublishing.com/index.php/Kne-Life/issue/view/62>) | pages 252-265

### Immunohistochemical Detection of Porcine Reproductive and Respiratory Syndrome Virus Antigen in Formalin-Fixed, Paraffin-Embedded Tissues with Correlation to Clinicopathologic Data

(<https://knepublishing.com/index.php/Kne-Life/article/view/1134/2908>)

Lavina Gracia G. Manzano

## Conference Paper

# ADDING OF L-ARGININ AMINO ACID IN SKIM MILK DILUENT TO MAINTAIN QUALITY OF BUCK SPERM in COLD TEMPERATURE

Tri Wahyu Suprayogy, Suherni Susilowati, and Tatik Hernawati

Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

## Abstract

Nowdays, the storage of buck semen in cold temperature have not satisfied yet because in buck's seminal plasma contains phospholipase enzyme which can coagulated egg yolk in diluents. The specific aim of this study was to investigate the benefits of L-Arginin amino acid in skim diluents to quality buck's spermatozoa on cold temperature. This research utilized four treatment groups, namely Controlled group (Po): skim milk diluent without L-Arginin + buck's semen; P1: skim milk diluents + L-Arginin 0,002M/ml + buck's semen; P2: skim milk diluents + L-Arginin 0,004 M/ml + buck's semen and P3: skim milk diluents + L-Arginin 0,006 M/ml + buck's semen. Then the samples stored in cold temperature (5°C). The result showed that sperm motility, viability and membrane integrity were significantly different ( $p < 0,05$ ) among the treatments. The conclusion of this study is adding of L-Arginin Amino Acid in skim milk diluents maintain motility, viability and membrane integrity buck's sperm.

**Keywords:** L-Arginin, buck, cold temperature, motility, viability and membrane integrity.

## 1. Introduction

Frozen semen is semen freezing process at the temperature of liquid nitrogen -196°C [1]. Semen freezing is separated by slow freezing, rapid freezing and ultra rapid freezing. Freezing process causes the damaging of membrane structure and function as well as the ability of sperm to survive [2]. The purpose of the freezing process is as perfect as possible to retain some properties of biological materials, especially in viability [3]. The main obstacle of this freezing process is occurrence of cell damage. The reason partly due to the dehydration process does not occur to form intracellular ice crystals that can damage cells. It can also be due to increase the osmolarity of freezing media that lead to convert cryoprotectant into toxic resulting physical damage by the formation of extracellular ice crystals, the toxicity of concentrated electrolyte

Corresponding Author:  
Tri Wahyu Suprayogy  
twsuprayogi@gmail.com

Received: 03 October 2017  
Accepted: 10 October 2017  
Published: 29 November 2017

Publishing services provided  
by Knowledge E

© Tri Wahyu Suprayogy  
et al. This article is distributed  
under the terms of the  
Creative Commons Attribution  
License, which permits  
unrestricted use and  
redistribution provided that  
the original author and source  
are credited.

Selection and Peer-review  
under the responsibility of the  
VMIC Conference Committee.



or the osmotic swelling [4]. The damage during the freezing steps are common in the plasma membrane and the nucleus of sperm.

Plasma membrane of sperm consist of lipid, protein and karbohidrat. A characteristic feature of biological membranes is the asymmetrical arrangement of lipids within the bilayer. The lipid composition of the plasma membrane of mammalian sperm is markedly different from, those of mammalian somatic cells so that in the process of freezing the lipid membrane of buck sperm easy stimulating then Lipid Peroxidation (LPO) occurred. Sperm cells containa high content of polyunsaturated fatty acids. The ratio of unsaturated to saturated fatty acids in small ruminant sperm membrane is also higher than in other species, making the membranes more susceptible to peroxidative damage in the precence of ROS-with a subsequent loss of membrane integrity in the acrosomal region, impaired cell function and decreased motility of the sperm [5].

L-Arginin is an amino acid which has important roles to stimulate spermatozoa motility under in vitro condition in mammals. L-Arginin also enrolls in antioxidant that produces nitric oxide from enzyme synthesis reaction so that reduces lipid peroxidation sperm membrane that occurs because free radicals when sperm interacted with oxygen directly  $\text{NO}^2$  is a biological molecules which has important roles in spermatozoa fisiology like sperm motility, interaction between sperm and ovum, and spermatozoa. Nitric Oxyde ( $\text{NO}^2$ ) enrolls in a mechanism of sperm defense to be formed in to reactive oxygen under freezing level which defense sperm motility and viability post-thawing.

Until now the process of thawing buck's semen freezing is have not satisfied, it is because the buck' seminal plasma contains egg yolk coagulating enzyme or called by phospholipase A and triglycerol lipase [6]. Therefore, researchers wanted to examine the addition of L-Arginin Amino Acid in skim milk diluents to quality buck' sperm in cold temperatures.

## 2. Material and Methods

### 2.1. Experimental Animals

Semen samples from three bucks (3-4 years of age) were use in this study. The bucks were maintained under uniform nutritional condition. Ejaculated were collected twice a week from the bucks with the aid of an artificial vagina, immediately after collection was brought to the laboratory, and semen parameters were assessed, including volume, pH, consistency, color and consentration of the semen. After bucks semen underwent microscopic and macroscopic examination (appiying prequalification that the percentages of sperm motility and viability should be 70% or above).

## 2.2. Semen Dilution

One hundred skim milk added with 100 cc aquadest, heated until 92-95° C and then colded until 20-27°C temperature and added Penicillin 1.000 IU/cc diluents and Streptomycin 1 mg/cc diluents too. Diluents was prepared divided into four groups. Controlled groups (PO) refers to diluents of skim milk + semen of buck's (without L-Arginin). Group I (PI) refers to diluents of skim milk + semen of buck's and L-Arginin 0,002 M/cc. Group II (PII) refers to diluents of skim milk + semen of buck's and L-Arginin 0,004 M/cc. Group III (PIII) refers to diluents of skim milk + semen of buck's and L-Arginin 0,006 M/cc. All of groups colded at 5°C and then examined sperm motility, viability and membrane integrity.

## 2.3. Semen Evaluation

Progresive sperm motility was assessed using a phase contrast microscope. The motility of sperm was analyzed by mixing the semen gently and placing a 10 µl drop diluents of semen on a slide covered with a glass cover slip from five selected representative fields. Samples were selected randomly from 3 fields, for a total of 100 cells. Individual sperm were recorded as being viable or dead [7].

Eosin negrosin staining was used to evaluate sperm viability as ceshcribed by Susilowati [7]. Semen was placed on glass slide and this samples was mixed with one drop of eosin-negrosin solution. The mixture was smeared on the the glass slide and allowed to air dry. One hundred of sperm were evaluated in at least five different fields in each smear under a light microscope. Eosin penetrates non viable cells, which appear red and negrosin offers a dark background for facilitating the detection of viable, non stained cells [7].

Membran integrity was determined using the hypo-osmotic swelling test (HOST) described by Susilowati [7]. A total of 100 µl of semen was mixed with one mL of hypoosmotic solution (containing 13,51 g of fructose and 7,35 g of sodium citrate in 1000 mL of distilled water. The mixture was incubated at room temperature for 30 min. Following incubation, 15 µl of the sample was placed on a slide, covered with a cover slip and observed under a light microscope. The sperm were categorized according to the presence or absence of a swollen tail. At least 100 sperm were observed and the results were recorded as percentages. The membrane integrity after HOST was clas-sified into two groups, normal sperm that displayed coiled tails and abnormal sperm without coiled tails.



TABLE 1: Macroscopic and Microscopic Examinations Buck Semen.

Indicator	Character
Volume (cc)	1,5±0,45
Consistency	Viscous
Color	Milky white
Odor	Typicaly
pH	7
Mass Movement	+++
Individual Movement (%)	Progresive (90±6,50)
Concentration	3975x10 <sup>6</sup> spz/ml
Membrane integrity (%)	87±6,55

## 2.4. Statistical Analysis

All data were expressed as the mean values  $\pm$  SEM. The statistical significances of the effects of sperm quality (motility, viability and membrane integrity) were determined by Anova, P-values  $\leq 0,05$  were considered to be significantly different.

## 3. Result

Fresh buck semen used in this research had undergone macroscopic and microscopic examinations. Macroscopic examination included examinations on buck semen volume, color, odor, consistency and pH. Meanwhile, microscopic examinations investigated spermatozoa mass motility, individual motility, concentration, and viability. The result of microscopic and macroscopic examinations was presented on Table 1.

The research shows that buck's semen stored has milky white colour, typical odor, viscous consistency, pH = 7, volume 1,5±0,45 ml, concentration 3975x10<sup>6</sup>, mass motility +++ (the motion formed big and many waves), individual motility generally semen volume will increase based on their age, body weight, changing of condition, reproduction organ health, and frequency of semen collection. Colour, consistency, and spermatozoa concentration has correlation with others. The percentages of sperm motility, viability, membrane integrity after adding L-Arginin Amino Acid on the cold temperature were show in table 1, 2 and 3. Sperm motility, viability and membrane integrity were significantly different ( $p < 0,05$ ).

TABLE 2: Percentage of buck's sperm viability in the skim milk diluents + L-Arginin Amino Acid which stored in cold temperature (5°C) and evaluated everyday.

Treatment	1 st day (%)	2 nd day (%)	3 rd day (%)	4 th day (%)	5 th day (%)
Po	71,67±2,73 <sup>c</sup>	64,50±5,32 <sup>c</sup>	57,00±5,40 <sup>c</sup>	48,00±6,23 <sup>c</sup>	41,17±4,87 <sup>c</sup>
PI	75,50±3,45 <sup>b</sup>	70,67±4,32 <sup>b</sup>	64,00±4,19 <sup>b</sup>	55,67±5,28 <sup>b</sup>	46,33±8,04 <sup>b</sup>
PII	75,50±2,17 <sup>b</sup>	70,50±3,15 <sup>b</sup>	64,67±4,59 <sup>b</sup>	58,00±2,28 <sup>b</sup>	50,00±3,03 <sup>b</sup>
PIII	80,58±7,24 <sup>a</sup>	72,54±8,02 <sup>a</sup>	75,50±3,56 <sup>a</sup>	69,00±3,92 <sup>a</sup>	59,50±3,21 <sup>a</sup>

Note: Value in the same row with different superscripts indicate significant difference at (p<0,05)

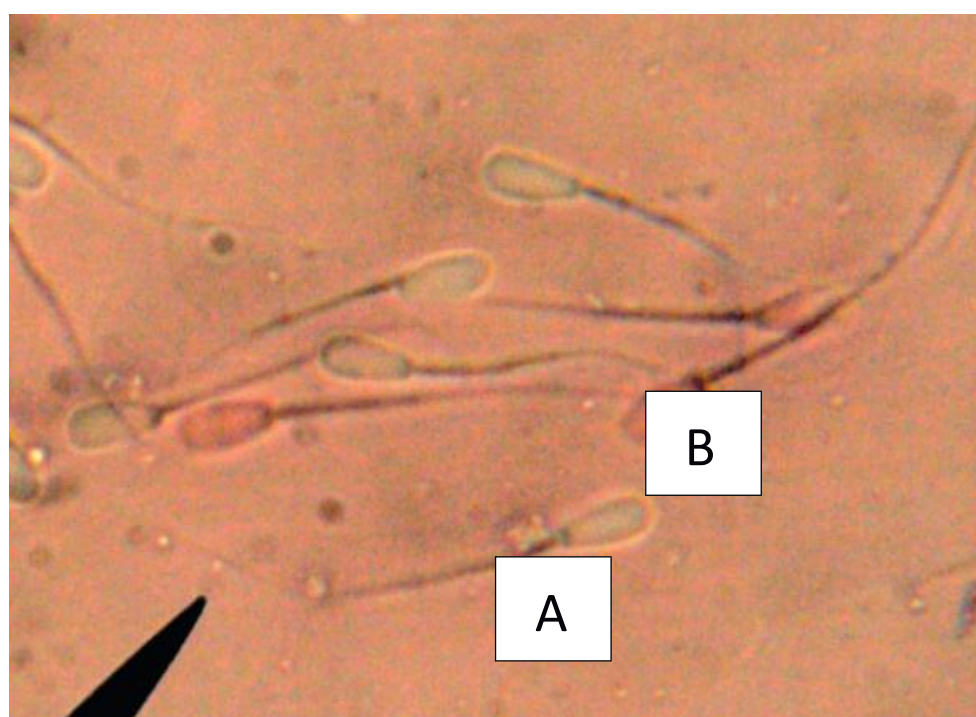


Figure 1: Live sperm (A. uncoloured head) and dead (B. coloured head).

## 4. Discussion

In this study motility, viability and membrane integrity after adding L-Arginin Amino Acid in skim milk in group III was higher compare with another group. Viability of sperm was conducted utilized through eosin negrosin coloration which was based on coloration permeability into spermatozoa. The mechanism of this process was the living spermatozoa are indicated by membrane integrity, although their environment is colorless (in this case reddish) the head of living sperm will remain colorless or transparent due to normal plasma membrane integrity, meanwhile dead sperm (due to damaged plasma membrane) could not control the permeating coloration substance resulting the head to appear reddish Plasma membrane didn't function in protecting

TABLE 3: Percentage of buck's sperm motility in the skim milk diluents + L-Arginin Amino Acid which stored in cold temperature (5°C) and evaluated everyday.

Treatment	1 st day (%)	2 nd day (%)	3 rd day (%)	4 th day (%)	(5 th day (%))
Po	61,50±3,08 <sup>d</sup>	56,17±4,42 <sup>c</sup>	50,50±5,24 <sup>c</sup>	44,17±6,08 <sup>c</sup>	36,83±6,08 <sup>c</sup>
PI	67,33±3,45 <sup>c</sup>	56,83±4,35 <sup>c</sup>	51,83±7,22 <sup>c</sup>	49,50±5,24 <sup>c</sup>	39,33±6,50 <sup>c</sup>
PII	73,50±2,17 <sup>b</sup>	69,50±3,50 <sup>b</sup>	62,17±4,75 <sup>b</sup>	55,33±3,23 <sup>b</sup>	46,17±2,64 <sup>b</sup>
PIII	78,00±2,28 <sup>a</sup>	75,17±2,28 <sup>a</sup>	75,50±3,56 <sup>a</sup>	65,67±3,50 <sup>a</sup>	57,33±3,72 <sup>a</sup>

Note: Value in the same row with different superscripts indicate significant difference at (p<0,05)

TABLE 4: Percentage of buck's sperm membrane integrity in the skim milk diluents + L-Arginin Amino Acid which stored in cold temperature (5°C) and evaluated everyday.

Treatment	1 st day (%)	2 nd day (%)	3 rd day (%)	4 th day (%)	(5 th day (%))
Po	31,00±2,83 <sup>c</sup>	28,33±2,16 <sup>c</sup>	25,07±2,87 <sup>c</sup>	22,83±2,79 <sup>c</sup>	17,83±2,63 <sup>c</sup>
PI	32,00±2,10 <sup>c</sup>	35,53±3,31 <sup>b</sup>	31,33±2,25 <sup>b</sup>	22,67±2,80 <sup>c</sup>	18,67±1,63 <sup>c</sup>
PII	38,67±2,16 <sup>a,b</sup>	36,50±3,27 <sup>b</sup>	31,50±3,33 <sup>b</sup>	25,17±4,17 <sup>b</sup>	22,83±3,92 <sup>b</sup>
PIII	40,80±4,38 <sup>a</sup>	37,17±2,93 <sup>a</sup>	34,50±4,23 <sup>a</sup>	28,33±6,08 <sup>a</sup>	25,50±5,57 <sup>a</sup>

Note: Value in the same row with different superscripts indicate significant difference at (p<0,05)

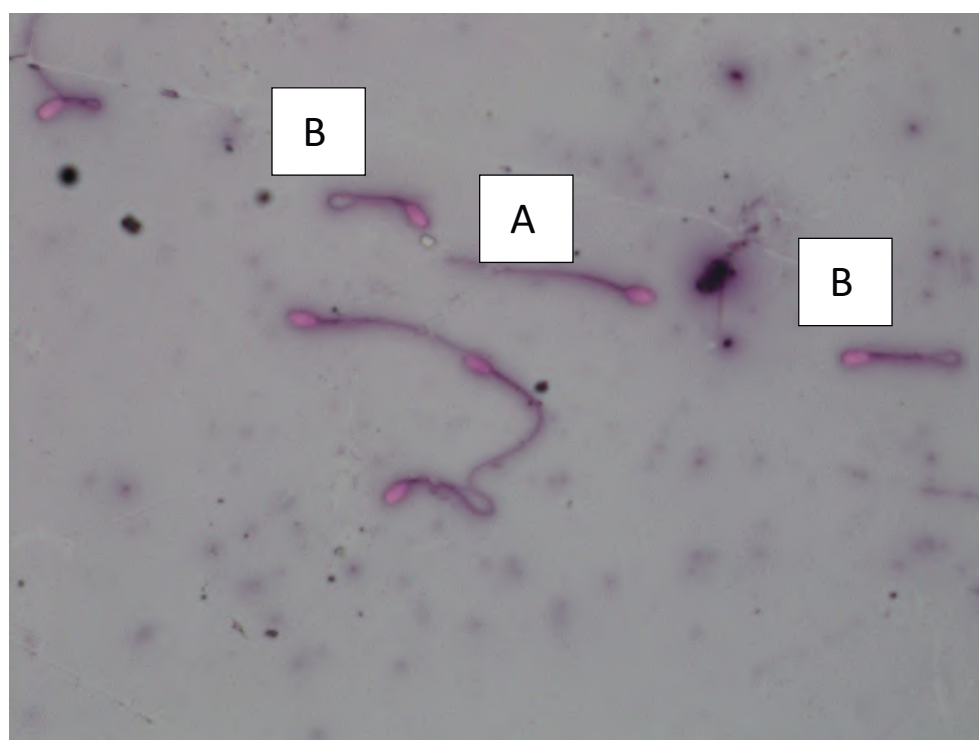


Figure 2: Damage membrane sperm (A. straight sperm tail) dan intact membrane sperm (B. round spermatozoa tail).



cellular organelles from mechanical damages, it also functioned as a filter which circulated and preserved intracellular substances on metabolism process [6]. Sperm motility correlated to spermatozoa ability to fertilize ovum. Spermatozoa movement was enhanced by energy (in form of Adenosine Triphosphate/ATP) produced by mitochondria and dynein motor (spermatozoa flagellum cytoskeleton whose movement was regulated by  $\text{Ca}^{2+}$  and cyclic Adenosine Monophosphate (cAMP) [11]. The energy was the result of oxidative phosphorylation of enzymatic processes happened in mitochondria. The enzymes which took part in oxidative phosphorylation process lied on mitochondria inner surface. Oxidative phosphorylation process produced free energy (which was converted into ATP molecules and Reactive Oxygen Species (ROS) compounds. As long as stored, motility of sperm would be decreased. This condition correlated in increasing  $\text{Ca}^{2+}$  in the cells so cAMP also decreased, beside causing by lactic acid form. As many as lactic acid formed would be decreasing pH., so metabolic process would be disturbed. Metabolic decreasing causing ATP formed decreasing and then sperm motility would be decreasing [6].

L-Arginine is amino acid which can stimulate sperm motility in the in vitro condition and also have role in cellular immunity defense [8]. L-Arginine such protect spermatozoa against lipid peroxidation through increased production of nitric oxide used against reactive oxygen species (ROS) such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and anion superoxide ( $\text{O}_2^-$ ) [9]. Antioxidants work by giving an electron to the free radical compounds. Antioxidants are able to activate the growth of the oxidation reaction, by preventing the formation of free radicals. Antioxidants can inhibit oxidation reactions by binding free radicals, that damage cells can be inhibited [10]. L-Arginine is secondary antioxidants which works by cutting the oxidation chain reaction of free radical or by catching free radicals, as a result of free radicals cannot react with cellular component. L-Arginine increases glycolysis spermatozoa so the process can produce energy in the form of energy the movement, so can remain motile and simultaneously maintain viability in the process.

## 5. Conclusion

Adding of L-Arginine Amino Acid can maintain progressive motility, viability and integrity membrane of buck's sperm in skim milk diluents on cold temperature.

## References

- [1] Direktorat Jenderal Produksi Peternakan, 2007. Petunjuk Teknis Produksi dan Distribusi Semen Beku. Departemen Pertanian. Jakarta
- [2] Lessard, C.,S. Parent, P. LEclerc, J.L. bailey and R. Sullivan, 2000. Cryopreservation Alters the Levels of the Bull Sperm Surface Protein P25b. *Journal Andrology*.21:700-707.
- [3] Supriatna, I dan Pasaribu,FH, 1992. In Vitro Fertilization. Transfer Embrio dan Pembekuan Embrio. Bogor. PAU.IPB.
- [4] Kasai, M. 1996. Simple and Efficient Methods for Vitrification of Mammalian Embryos. *Animal Reproduction Sciences*. 42(1): 67-75.
- [5] Alvarez, J.G and BT Storey, 1995. Differential Incorporation of Fatty Acid Into and Peroxidative Loss of Fatty Acid from Phospholipid of Human Spermatozoa. *Mol. Reprod.Dev*. 42: 334-345.
- [6] Garner and Hafez, E.S.E, 2000. *Reproduction in Farm Animal*. 7<sup>th</sup> Edition. Philadelphia. Baltimore. New York London.
- [7] Susilowati,S. Potensi Biologis Insulin Like Growth Factor-I Complex Plasma Seminalis Kambing Hasil Sentrifugasi. Disertasi. Pasca Sarjana Fakultas Kedokteran Universitas Airlangga.
- [8] Srivastava,S., Prashant D., Evans C., Girjesh G. 2006. Mechanism of Action of L-Arginin on The Vitality of Spermatozoa is Primarily Through Increased Byosynthesis of Nitric Oxide. *Biology of Reproduction* 74: 954-958.
- [9] Al-Ebady,AS., SO. Hussain, KI.,Al-Badry and FF. Ibrahim.2012. Effect of Adding L-Arginin on Some Parameters of Bull Sperms After Freezing in Liquid Nitrogen (-196oC). *Al-Qadisiya Journal of Vet Med Sci*. 11(2):15-161.
- [10] Winarsi, H. 2007. *Antioksidan Alami dan Radikal Bebas*. Kanisius. Yogyakarta.
- [11] Yanagimachi, 1994. *Mammalian Fertilization*. In : *The Physiology of Reproduction*. E. Knobil, J. Neil, L.L. GS. Greenwald, C.L. Market, DW. Plaff. Raven Pers LTD. New York.